US Army Medical Research and Materiel

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Assay for the Proteolytic Activity of Serotype A from *Clostridium botulinum*

This invention provides an enzymatic assay which allows different preparations of botulinum toxin (BNT) serotype A (BNT/A) to be conveniently quantitated, standardized, and compared on the basis of specific enzymatic activities.

BNT/A is one of seven serologically-distinct, highly-potent neurotoxins produced by various strains of *Clostridium botulinum*. BNT/A has beneficial applications; it is a useful tool for research on the mechanisms of neurotransmitter release and is used as a drug to treat a variety of human muscle dysfunctions. However, conventional methods for estimating BNT concentrations, such as the mouse lethality assay and an antibody neutralization test, require the use of animals, can take up to four days, and are not universally standardized. Additionally, different results can be obtained depending on which protocol is used. Comparison of different BNT lots or BNT prepared in different laboratories is difficult. Better and more reliable methods are therefore needed.

The assay can be used to standardize toxin lots and track the toxin during production and purification of BNT/A containing drugs or solutions. It could be adapted to [illegible] hundreds of compounds at a time for toxin inhibitory activity. The antitoxin effectiveness of down-selected compounds for treatment of botulinum intoxication could then be tested further. The assay could also potentially screen for compounds that stimulate BNT/A activity which could be used to treat human muscle [illegible]tions.

Features and advantages:

- **Can perform in one hour or less, without lab animals**
- **Measures proteolytic activity and thus may replace the mouse lethality assay**
- **Uses fluorescamine as the detection reagent**
- **Expensive, time-consuming product separation step unneeded as non-reactive with fluorescamine**
- **Could provide a method for screening compounds for BNT/A**

Patent Status

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